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THE BLACK-SHANK OF TOBACCO IN PORTO RICO

by

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MELVILLE T. COOK, EDITOR

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THE BLACK-SHANK OF TOBACCO IN PORTO RICO [1]

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Following a definite policy assumed by the Insular Experiment Station relating to a detailed study of tobacco diseases, a survey of the most important tobacco regions was made in the fall of 1926. Among other maladies a serious disease known among the laborers in tobacco fields as "pata-prieta" and similar to or the same as the American black-shank, was found on a type of commercial cigar wrapper tobacco. No studies had been made in Porto Rico prior to 1926 on this important malady. Although the growing of the cigar wrapper tobacco was quite an industry in the island at the time our investigations were begun it has now disappeared. Other factors aside from the disease, have been responsible for this failure of a once flourishing industry. It is not our purpose to discuss them at this time. But suffice it to say that the question of the variety and type has been a dominating factor. Let better types of tobacco which may be adapted to our conditions of soil and climate such as exist today be developed and safeguarded from degeneration, and the growing of cigar wrapper tobacco will again assume the proportions it once held. Further, the black-shank has also been found in certain localities on cigar filler types and its prevalence in fields of this tobacco might increase in the future. It seems proper to give the results of our studies on this disease in Porto Rico at this time.

PLANTS AFFECTED

The pathogene (*Phytophthora nicotianae* Breda de Haan) which causes black-shank of *Nicotiana tabacum* L. has been reported by Breda de Haan (2) as attacking Amaranthus sp. and Androng weed in Java, while Palm (8) found the castor oil plant (*Ricinus communis* Linn), the tomato (*Lycopersicum esculentum* Mill.) and *Commelina nudiflora,* to be susceptible and the potato (*Solanum tuberosa* L.) not susceptible. He also found *Trema amboinensis* susceptible, under certain conditions. Tisdale and Kelly (12) found

[1] This investigation was made in cooperation with the Porto Rican Leaf Tobacco Company, the only growers of cigar wrapper tobacco in Porto Rico.

that tomato, potato, the castor oil plant and eggplant (*Solanum melongena* L.) would develop disease symptoms when inoculated.

In our experiments we have tested the castor-oil plant, potatoes, peppers and eggplant. The pathogene causes the wilting of the leaves of eggplant inoculated at the axils and also death of terminal buds in plants which are beginning to form the first flower bud. Eventually, it kills the whole plant. Potato stems have been inoculated and death of the tissues has been produced by the fungus but lesions are localized and limited in extent. Damping-off, black-shank and leaf-spot have been produced on *Ricinus communis* seedlings, growing on infested soil. Some plants transplanted into infested soil did not show the disease; but later on, at blossoming time, upon being dug out, they showed a general blackening of the epidermis and cortex of roots and underground part of stem from the tissues of which the fungus was recovered. The fungus also produces "damping-off" or "bending-off" of eggplant, tomato and pepper seedlings under favorable conditions.

Varietal Susceptibility.—Tisdale and Kelly (12) tested 18 cultivated varieties of *Nicotiana tabacum* and the percentage infection ranged from 90 for Big Cuba (Type E) to 100 for 12 of the remaining types and varieties. The remaining five types and varieties ranged between 95 per cent for a Porto Rican type to 99.5 per cent for Commercial Broad Leaf. *Nicotiana rustica* showed 30 per cent infection in a population of only 54. They consider *N. rustica* showing this percentage of infection as a "highly resistant variety to the disease". As will be seen later in our studies we have not considered a variety to be highly resistant which shows as low as 26.08 per cent diseased individuals. That would be about one-fourth of the crop and, certainly, growers would be risking too much when growing varieties which might contract the disease to such an extent. Tisdale (11) reports that five varieties of bright or flue-cured tobacco tested were found to be "highly susceptible".

In Porto Rico natural field infections occur most virulenty and aggressively on the "Borinquen" (Connecticut Round Tip) tobacco, a cigar wrapper type imported from the United States and grown extensively by the Porto Rican Leaf Tobacco Co. of Porto Rico up to 1927. Field observations and laboratory studies have proved this variety to be the most susceptible of all wrapper varieties of tobacco in Porto Rico. Cases where 80 per cent of the crop was affected have not been rare. In an experimental plot at the Experiment Station grounds about 100 per cent infection was recorded. A type of cigar wrapper tobacco selected and improved in the island from

its own stock has proved very resistant, in fact almost immune. A field of tobacco of this variety grown side by side with a field of the Connecticut Round Tip variety demonstrated, beyond all doubt, that the Porto Rican type was very resistant. Counts were made of the Connecticut Round Tip field which showed about 40 per cent infection as contrasted with no diseased individuals in the adjoining field of the Porto Rican variety. The fact that the two varieties were grown in separate fields detracts from the value of this count and naturally raises doubt as to whether the second field where the apparently resistant variety was grown was infested with the causal organism. However, the two plantations were separated only by a narrow path and irrigation ditches ran up and down both fields, with all the chance for the inoculum to be constantly carried from the diseased field to the healthy one. Frequent talks with Dr. G. H. Chapman, until recently Field Manager of the Porto Rican Leaf Tobacco Co., and other members of their field force, showed that the disease had been severe on the Connecticut Round Tip in previous years where the Porto Rican type was growing in 1926–27.

As an introduction to our studies on varietal susceptibility an attempt is here made to describe briefly all the varieties and strains with which we have dealt. Mention has already been made of the "Borinquen" (Bor) and Porto Rican wrapper varieties. A mammoth variety designated as *Experiment Station* (*Expt. Sta.*), was obtained from a gardener at the Experiment Station, who discovered it by mere chance in one of the cutting propagation beds. This plant, because of its high susceptibility to black-shank, has been very useful in our investigations.

The "Ceniza" (Cen) variety is a well-selected native cigar filler tobacco. This and the variety "Virginia Blanco" (probably the best Porto Rico cigar filler) have been selected and propagated by Mr. F. H. Bunker, Tobacco Expert of the Insular Department of Agriculture, during the last few years. They are the most sought for varieties in the island today.

The variety *Consolation* (Con.) is a yellow-leaved cigar wrapper variety which appears to be a mutant from one of the Porto Rican cigar filler varieties. It was grown extensively for wrappers in the Cayey-Aibonito district until 1927 when it was discarded.

"Vuelta Abajo" (V), is a variety imported from Cuba, and grown for wrapper in 1926–27. Its culture has been abandoned.

The strain J–18 was developed by Dr. G. H. Chapman from "Virginia Blanco".

"Magnolia" is a Cuban tobacco grown to a small extent for filler in 1926–27 and for both filler and wrapper purposes in 1927–28.

"Gigante" is a semi-mammoth tobacco grown in the Cayey district. It is of the filler type.

"País".—Under this name are included a number of Porto Rican filler varieties or strains, most of which are of doubtful economical value.

The following data were gathered in a plantation on relatively high land. The two varieties "Ceniza" and "Virginia Blanco" were grown in the summer of 1927 for seed by the Porto Rican Leaf Tobacco Co. in Caguas. Black-shank appeared in late summer. Counts were made early in the season of all the healthy plants. Diseased plants were pulled out at intervals. At the end of the season counts were made of the healthy plants.

The results are given in the following table.

TABLE No. I

Incidence of Disease in Two Varieties of Commercial Filler Tobacco

Variety	Population	Diseased-individuals	
		Total number	Percent
"Virginia Blanco"	4,663	749	16.06
"Ceniza"	464	121	26.08

The differences in population in the two varieties does not allow of a reasonable comparison. Yet, it is clear that these varieties are not so highly resistant as had been generally supposed in the island. The "Virginia Blanco" with 16.06 per cent infection should be considered as fairly resistant.

The *Consolation* variety is quite resistant to black-shank. Plants grown in pots with infested soil have not developed symptoms except in a few cases. That this would be its behavior in infested fields has not been ascertained. It was not further tested since it is no longer of commercial importance. The type "Vuelta Abajo" has shown high tolerance to black-shank. Inoculations in pots nearly always produce symptoms of the disease although the plants are able to survive while similarly inoculated varieties like "Borinquen", for instance, readily succumb to infection. "Magnolia" tobacco is quite susceptible. Both field observations and inoculations show this to be true. "Gigante", in comparison with "Ceniza" shows to be as susceptible as the latter. All the types "País" have not been tested. There is only one type obtained from the Porto Rican

Leaf Tobacco Co. which has been planted in our grounds and inoculated with the black-shank pathogene. All inoculated plants became infected but a number of them were able to reach complete development. Plants received from various places in the tobacco sections including two specimens from the coastal plain (Bayamón), upon examination showed the causal pathogene present in the lesions at the base of the stem. Plants received from Jayuya in May showed the disease.

In the following table are given the results of counts of diseased plants in a number of strains, varieties and first generations of crosses.

TABLE No. II

Incidence of disease in different varieties and arrangement of rows in the field. The varieties J–18 (rows 60–72), Ceniza (rows 73–82) and *Experiment Station* (rows 83–85) on one end of the field grown with no shade, all other varieties and crosses under cloth.[1]

Variety, strain, cross.	Row No.	Population	Diseased plants	
			Total number	Percent
Con. x V. (1st gen.)	1–2	151	2	1.32
V. x Con. (1st gen.)	3–4	170	3	2.35
V. x Cen. (1st gen.)	5–6	161	6	3.73
Cen. x V. (1st gen.)	7–8	172	6	3.43
V. x Exp. Sta. (1st gen.)	9–10	167	85	50.89
Exp. Sta. x V. (1st gen.)	11–12	182	128	70.33
Bor x V. (1st gen.)	13–14	178	44	24.72
V. x Bor (1st gen.)	15–16	129	55	42.64
Con x Exp. Sta. (1st gen.)	17–18	178	34	19.10
Exp. Sta. x Con. (1st gen.)	19–20	183	28	15.30
Cen. x Exp Sta. (1st gen.)	21–22	173	96	55.49
Exp. Sta. x Cen. (1st gen.)	23–24	180	142	78.89
Bor x Exp. Sta. (1st gen.)	25–27	208	208	100.00
Exp. Sta. x Bor (1st gen.)	28–29	173	173	100.00
Exp. Station	80–82	262	262	100.00
P. R. R. Gr. 3	33–35	246	3	1.22
P. R. R. Gr. 8	36–38	257	9	3.50
P. R. Ind. 6 P. F.	39–41	247	4	1.62
P. R. Ind. 7 P. F.	42–44	255	12	4.70
P. R. Ind. 1	45–47	257	8	3.11
P. R. Ind. 2	48–50	279	8	2.87
P. R. Ind. 3	51–53	284	19	6.69
P. R. Ind. 4	54–56	279	17	6.09
P. R. Ind. 5	57–59	275	5	1.82
J–18	60–72	1,190	18	1.51
Cen. (Ceniza)	73–82	908	44	4.85
Exp. Station	83–85	269	260	92.94

Types P. R. R. Gr. 3 and 8 as well as P. R. Ind. 1–7 are all individual selections from the previously mentioned Porto Rican variety, made by various members of the field force of the Porto Rican Leaf Tobacco Co. P. R. R. Gr. 3 and 8 come from one plantation (Río Grande plantation) and P. R. Ind. 1–7 from another,

[1] Data of population was taken as soon as the transplants showed a vigorous growth, thus eliminating those plants killed by insects during the first few weeks. The data on diseased individuals was gathered at intervals and a final count of plants standing healthy at blossoming time was made to check with the figures on diseased plants already collected.

the Industria Plantation. The latter were selected by Mr. Pedro Ferrer and the former by Dr. G. H. Chapman.

In general, the results given above show all the P. R. selections to be highly resistant to black-shank. The most resistant strain seems to be P. R. R. Gr. 3 with only 1.22 per cent infected plants and the most susceptible, the P. R. Ind. 3 with 6.69 per cent infection. The populations are not equal in the two cases.

It might be well to consider the results of these selections in two sets, the P. R. R. Gr. on the one hand and the P. R. Ind. on the other. P. R. R. Gr. 3 and 8, together make a population of 503 with 12 diseased individuals and a percentage of infection of 2.39, P. R. Ind. 1–7 together make a total of 1,876 plants with 73 diseased ones and a 3.89 per cent infection. Since both sets of selections were made from the same variety namely, P. R. wrapper, it is well to collect the results into one group. Thus, the nine varieties would represent a population of 2,379 individuals with 85 diseased ones among them. The percentage infection for the variety would then be 3.57. A variety with such a low percentage of susceptibility should be considered very resistant, especially if compared with the variety *Expt. Station* grown in the same field and in adjacent rows and showing an almost complete susceptibility—92.94 and 100 per cent infection.

First generations of direct and reciprocal crosses between Borinquen and *Expt. Station* (the two types which are very susceptible) showed a similar high susceptibility—since all the plants died. No seed could be saved for a second generation. The first generation population of direct and reciprocal crosses between "Ceniza" and *Expt. Station* showed high susceptibility, yet one of the parents ("Ceniza") showed only 1.54 per cent infection in the same field. *Expt. Station* and Ceniza showed 78.89 per cent of diseased plants while Ceniza and *Expt. Station* had 55.49 per cent infected individuals. The population was about the same for the two cases. The first generation crosses of resistant types showed a high degree of resistance under the conditions in which the susceptible type *Expt. Station* failed and where the crosses between susceptible types showed high susceptibility. The results of counts of diseased plants in direct and reciprocal crosses indicate a lower percentage of infection in first generation plants of crosses between a resistant and a susceptible parent. *V* (Vuelta Abajo) and *Con.* (*Consolation*) are resistant types; while *Bor* (Borinquen) and *Expt. Station* are very susceptible. In the cross *Con.* × *Expt. Station* the percentage of infection was lower than in the cross *V* × *Expt. Station* which was again lower than the infection in *Bor* × *Expt. Station.* These results would

tend to show the higher resistance of each type predominating in the first generation. They are in line with the relative resistance of each individual type. As will be shown later these types are arranged according to their resistance to the disease as follows: "*Consolation*", "Vuelta Abajo", etc., and lower down in the list is "Borinquen".

In the same table the results are given of three varieties grown without shade but in the same location as those already discussed.

The J–18 strain showed an infection of 1.51 per cent as compared with 4.85 per cent for "Ceniza". The *Expt. Station* variety here again displays its high susceptibility with about 92.94 per cent diseased individuals. The fact that the *Expt. Station* type showed a lower percentage of infection—only 7.06 less—when grown without shade may be due to the fact that the land dries out more quickly when unshaded. Further, it should be stated here, that these fields were subject to very high humidity at intervals. They were under water thrice and in each case the water remained in the ditches for a few days. These last three varieties grew on a slightly higher land than the other varieties which appear in the table.

It is evident that the "Ceniza" variety is very resistant to the disease as is also true of the strain J–18. *Expt. Station* is by far, the most susceptible of any of the varieties tested. First generation populations of crosses between this and "Borinquen" and "Ceniza" are very susceptible to the attacks of the black-shank.

Summarizing our observations and results we are tentatively grouping the varieties of tobacco of any economic importance in Porto Rico according to their degree of resistance to black-shank as follows:

Most resistant:
1. Puerto Rico, cigar wrapper type.
2. "País", cigar filler type.
3. *Consolation*, cigar wrapper type.
4. J–18, cigar filler type.
5. "Vuelta Abajo", cigar wrapper type.
6. "Virginia Blanco", cigar filler type.
7. "Ceniza", cigar filler type.
8. "Gigante" (semi-mammoth) cigar filler type.
9. "Magnolia", cigar wrapper and filler type.
10. "Borinquen", cigar wrapper type.

Most susceptible:
11. *Expt. Station* (mammoth) not a commercial type.

It has already been pointed out that even the highly resistant types when inoculated will, in most cases, become infected, the dif-

ference between them and the very susceptible ones then being in their ability to tolerate infection. Infection in the former has not shown as lethal effects as on the susceptible types.

The Disease

NAMES

What is probably the same disease as the American black-shank is called "Lanas" or "bibit" in the Dutch East Indies. In Porto Rico it is generally called "pata-prieta" by the laborers and some growers. This term is an attempt at the translation of the English "black-shank". It probably originated when some one who was familiar with or had read about the disease in the United States tried to apply some name to what appeared to him as the same disease described from that country. Sometimes one hears foremen in tobacco fields call it "Faitapia". The Spanish pronunciation for this word faintly sounds to the ear like Phytophthora; and probably that is the way the word Phytophthora sounds to the non-English-speaking persons who hear the name from some American field manager. Another trouble caused by the same fungus in transplants is called "hinchado" (swollen) by the farmers, because the seedlings seem to become swollen at the base of the stem.

HISTORY AND RANGE

The first pubished report of the disease from Porto Rico appears to have been made in 1924 by Dr. Mel. T. Cook (3). He states that a shank disease caused some losses in the field. He does not give the cause of the disease. However, personal talks with members of the staff of the P. R. Leaf Tobacco Co., who have been working in the shade tobacco fields for a number of years, have convinced the writer that the malady existed in the island many years prior to 1924, although it seems probable that it was introduced from abroad. The disease had not been found on the sun crop tobacco varieties to any large extent. A few specimens of these types were received by the author in the fall of 1926 and they showed the characteristic symptoms of black-shank. That they were attacked by the pathogene responsible for black-shank was confirmed by laboratory studies. Later in the summer of 1927 it was noted that the disease was spreading on the cigar filler types and sporadic cases were found in Caguas during the months of January and February.

In the United States what is in all probability the same malady is supposed to have been introduced from some other country, Tisdale and Kelley (12). The disease probably did not exist before 1915

in the Florida-Georgia district. It has been spreading there since that year. Subsequently, it has been reported from Alabama in 1924 (Tisdale and Kelley) and in the same year from Virginia by Wingard and Godkin (13). Breda de Haan (2) reports its existence in Java since the year 1895. Horne in 1909 (5) reported a wilt disease of tobacco from Cuba, which, judging from his description, inoculations and statement that a fungus similar to that causing damping-off in the seed beds was found in the affected parts, (*"Un hongo aparentemente el mismo que causa la pudrición en los semilleros, ha sido hallado sobre las partículas descompuestas."*) is probably the same we have been studying.

IMPORTANCE

Were the shade tobacco industry to be revived in Porto Rico, measures would surely have to be taken to grow varieties which would resist or tolerate the "pata-prieta" or black-shank disease. The Connecticut Round Tip variety aside from other undesirable qualities, has the great disadvantage of being very susceptible to black-shank. There were heavy losses produced on fields of this variety in recent years. The return must then be made to other types. Yet under certain conditions even the now resistant varieties, might, if selection is not constantly made, gradually become more susceptible. Losses are heavier during seasons of heavy rainfall and floods.

In the seedbeds the losses have increased from year to year. The disease occurs in the seedbeds both in the form of damping–off and a leaf-blight. Our observations and experiments show that the form of damping-off and the seedling blight produced by the black-shank pathogene are more injurious than the damping-off produced by the fungus *Pythium debaryanum* Hesse. In the fall of 1927 more than 50 per cent of the seedbeds were destroyed by the damping-off caused by the black-shank and the latter fungus. As a result of shortage of plants there was a short crop of tobacco that year. We are convinced that the black-shank fungus will more and more be a dominating factor in the production of tobacco seedlings. Tisdale and Kelley (12) report no direct injury to seedlings in the Florida-Georgia district. It causes injury however in seedbeds in Java (2).

Black-shank has been supposed to be harmless to filler types of tobacco by various growers. The data offered in the preceding paragraphs on varietal susceptibility show that this is not the case. That filler types are also liable to contract the disease normally in the field is further proven by cases observed during December, January and February in the Caguas district. Upon the suggestion of Mr.

Nelson Márquez, Deputy Inspector of Agriculture in that district, the writer visited a mixed planting of "Virginia Blanco", "Ceniza" and "Borinquen" varieties where we found plants of all these varieties with typical black-shank or "pata-prieta" symptoms. The "Borinquen" plants probably came as a mixture in the filler types. Most of the plants of this variety ("Borinquen") were diseased. Other similar cases of black-shank in filler types were observed in low, level lands near streams.

Plants received in May from Mr. F. González, Agricultural Agent at Jayuya, showed the typical symptoms of "pata-prieta" or black-shank. Laboratory studies showed the presence of the pathogene which causes this disease. The time may not be far distant when these sporadic infections may become more generalized in the commonly grown commercial filler types, as has been the case in the shade tobacco.

SYMPTOMATOLOGY

Morphologic symptoms.—The malady "pata-prieta" or black-shank derives its name from the most characteristic external manifestation of the disease, a blackening of the lower or basal portion of the plants. Roots are also affected. There are many cases in which plants wilt and die as a result of the destruction of the roots before any blackening of the stem has appeared above the surface of the soil. The lesions in the roots occur as small brownish spots which soon develop into long necrotic brown, hazel or black areas extending all the way down or up the root and involving other neighboring roots. Thence infection moves beyond the root system and into the stem. Usually only a number of the secondary roots are affected. Still there are other cases in which the black necrotic areas have extended high up the stem without much destruction or blackening of the roots. In these cases the infection probably occurred at the base of the stem. The disease starts in the stem at the surface of the soil, if conditions are favorable, and it rapidly advances up the stem and down the roots. The stems of large plants may show lesions a foot or more above the base, but usually not more than six inches in length. All plants thus affected rapidly wilt, hence the name "sueño" (sleep), applied to it by the laborers. Then yellowing, shriveling and finally browning of the lower leaves ensues, especially if a dry period follows infection. Gradually the majority of the leaves take on this appearance.

In plants which become infected when only about a foot or less in height, the disease, if weather relations are favorable, advances from roots to stem and up to the growing bud, the entire stem taking

on a black appearance, shriveling and falling to the ground. The foliage, whose blades are still green for about half of their length, gives the apperance of leaves arranged radially and alternately in a circle on the soil.

On the leaves the pathogene produces spots which are at first circular in outline then changing to irregular blotches or patches. These are at first pale greenish or olivaceous, later pale or creamy in the central portion immediately surrounded by a light brownish discoloration, limited by the pale green zone near the healthy tissues. The pale color develops only when dry weather follows infection. If the atmosphere is humid the fungus rapidly extends from the point of inoculation and produces a rotting of the blade. This rotting follows its way through the vascular bundles into the petiole and thence into the stem. The symptoms on the leaves of large plants are similar in every respect to those produced on seedlings in the seedbeds. The spots occur mostly on the "sand" or lower leaves but may also appear on the lower middles. The fungus penetrates hairs and causes them to shrivel.

A fungus which was found to be the pathogene which causes the disease under discussion was isolated from transplants which failed to develop a root system. The seedlings were transplanted during the heavy downpours of late October and early November in Cayey. This trouble extended over an area of 32 acres in three different farms. The leaves remained apparently healthy, but no progress was made by the transplants. Upon examination of such plants it was found that new roots failed to appear, the stem below the surface of the soil had become slightly swollen and of a dirty white or very pale green color. There was a slight discoloration of the tissues below the cortex.

The disease produces small, lens-shaped to elongate, brownish to black lesions on the stems of large seedlings while still in the seedbed; these do not seem to affect the plant much in the beds except for the stimulation of adventitious roots. This root development is probably induced by the fungus infection. The lesions do not cause the death of such seedlings in the bed but when they are transferred to the field the fungus under favorable conditions then produces a general infection resulting in the death of the plants. If the weather is dry, however, the plants may recover.

The damping-off phase of the disease will be discussed in greater detail in another publication.

The symptoms on *Ricinus communis* seedlings are as follows: (*a*) on the leaves the spots which are at first olivaceous in color

later become pale yellow or creamy. They enlarge and gradually cover the whole blade of the young leaves. This disease follows its course down the petiole to the stem which it destroys to a distance of about ¾″ below the axil. In few cases it destroys the whole seedling. The leaves shrivel up and die. New leaves may develop when the disease is limited to the cotyledon leaves.

(*b*) On stems when inoculation has taken place about ½″–1″ from the surface of the soil on seedlings about 2½″ high, the first symptom is a change in color from an ashy green to a brownish black. The affected tissues shrink. The lesions enlarge both laterally and longitudinally and may reach the growing tip.

(*c*) On the roots. Invasion is not infrequent through the roots. The primary root is usually the first one to show any symptoms. Lesions appear on the roots as brownish spots which gradually extend over the whole root and into the stem.

Signs: Conidia and sporangia are produced in abundance on the surface of stem lesions near the soil. The pathogene as a saprophyte in the soil and in dead roots also fructifies heavily and it is probably in this phase that the majority of the spores which go to produce secondary cycles are formed. They are also found at the end of mycelial threads on the surface of spots on leaves, in particular, during rainy weather. Chlamydospores are very common and may be found in the tissues of the leaves, stem and roots or in the soil. Oospores are produced by the fungus but not in abundance. They are formed in the old dead roots, stems and leaves and in greater numbers in the soil in debris. Zoospores are found in large numbers in low places where the water collects. Such water if examined in the morning will be found to contain sporangia germinating and liberating their zoospores.

Histologic symptoms. The symptoms are characteristically of the necrotic type. Soon after penetration of the pathogene the first apparent symptom is hydrosis, followed by discoloration of the primary walls of epidermal and subepidermal cells. The hyphae are both intra and intercellular, generally intracellular. They penetrate into the vascular bundles and may be found going across the bundles through phloem and xylem. The cortex is usually affected, the hyphae causing the dissolution of the parenchyma cells. These gradually shrink under the drying and sinking epidermis.

The hyphae seem to encounter no barrier in the tissues that would check their progress, such as is reported by Braun (1) for *Pythium complectens* Braun which causes geranium stemrot, etc. The parenchyma cells of the pith are sometimes also affected. In the early

stages they become stained brown, olivaceous or greenish, their primary walls are dissolved and they begin to lose their turgidity. Later stages show a shrunken black, dry pith.

ETIOLOGY

Name, history and classification of the pathogene.—The pathogene which causes "pata-prieta" in Porto Rico is probably the same as that causing black-shank in the United States and "Lanas" or "bibit" in the Dutch East Indies; namely, *Phytophtora nicotianae* Breda de Haan. The fungus was described in 1895 by J. van Breda de Haan (2). Tisdale and Kelley (12) have found that there were slight differences in morphological and physiological characters between the pathogene from America (Florida-Georgia) and *P. nicotianae* from Java.

In our studies we have endeavored to compare our cultures with the pathogene from Forida and that from Sumatra and have carried in comparison a Phytophthora isolated from tomato seedlings as a check. Comparison with other species of Phytophthora did not seem justified as similar investigations have been already made by Tisdale and Kelley (12). The cultures used in this investigation were as follows: P1, isolated from diseased seedlings, Cayey, P. R.; P3, isolated from diseased seedlings, Cayey Model Farm; P4, P10 and P14, isolated from diseased plants from the field, Caguas, P. R.; P16, (*Pythium de Baryanum*,) from tobacco seedlings, Caguas, P. R.; P17, tobacco transplant disease, Cayey, P. R.; P18, *Phytophthora* sp. isolated from rotting tomato seedlings, Río Piedras, P. R.; P20, (*Pythium de Baryanum*) from cucumber seedlings, Río Piedras, P216 (*Phytophthora nicotianae*, the Florida black-shank organism), obtained from C. M. Tucker of the Experiment Station at Mayagüez, P. R., who secured it from Tisdale of Florida; and a culture from Sumatra also obtained through the courtesy of Mr. Tucker.

The morphology of our cultures was critically studied and compared with that of *Phytophthora nicotianae* from Florida. Unfortunately, the Sumatra culture of this pathogene did not sporulate well and not as many reproductive bodies were observed, as were desirable. However, since Tisdale and Kelley (12) have already compared their pathogene with what they call strains of the fungus from Holland and Java, we feel justified in drawing conclusions from comparisons with the Florida black-shank pathogene.

Measurements of length and width of sporangia were made from 14-day-old oatmeal agar cultures. Table III contains the lengths of various populations.

TABLE No. III

Lengths in microns of sporangia of cultures of *Phytophthora nicotianae* (P1, P3, P17, and P216) and of an undetermined species of Phytophthora from tomato (P 18).

Culture	Population	Maximum	Minimum	Mode	Mean
P1	277	58.65	27.60	44.85	46.653±0.230
P3	283	86.25	27.60	48.30	49.822±0.319
P17	206	65.55	20.70	48.30	47.197±0.385
P216	203	75.90	20.70	37.95	42.799±0.388
P18	145	69.00	25.43	41.40	47.359±0.539

It appears from the above table that no safe conclusions can be drawn from differences in lengths of sporangia of our cultures (P1, P3 and P17), the Florida black-shank pathogene (P216) and the Phytophthora from tomato (P18). It appears that cultures which agree on minimum length show different upper limits. The mean length of our cultures varies from 46.653 ± 0.230 microns to 49.822 ± 0.319 microns, while that for the Florida pathogene is 42.799 ± 0.388 microns. Tisdale and Kelley (12) gave the measurements for length of the sporangia on oat meal agar as 34.44 microns which is considerably lower than ours for the same pathogene.

The check, P18, shows a mean length almost equal to that of our P17.

From mean lengths alone one would be inclined to regard the Porto Rican fungus (P1, P3, P17) as constituting a strain different from P216, since the differences are quite significant.

A similar state of things is encountered with respect to widths of sporangia. Dimensions of widths were recorded in the same manner as, and simultaneously with the lengths. Table IV shows the widths of sporangia.

TABLE No. IV

Widths in microns of sporangia of cultures of *Phytophthora nicotianae* P1, P3, P17, and P216) and of an undetermined species of Phytophthora from tomato (P 18).

Culture	Population	Maximum	Minimum	Mode	Mean
P1	277	51.75	24.15	34.50	38.754±0.246
P3	283	55.20	20.70	37.95	39.291±0.271
P17	206	51.75	17.25	41.40	39.177±0.342
P216	203	51.75	17.25	30.05	33.834±0.331
P18	145	46.58	17.25	30.05	35.811±0.351

In the above table P216 shows minimum and maximum widths of sporangia equal to those of P17 but with a much lower mode, from which it appears that fewer spores had a small diameter in P17 than in P216. The population was about the same in the two cases.

Other results of maximum and minimum width comparisons with the remaining cultures do not mean much in this case. It is significant, however, that three of the *P. nicotianae* cultures had an equal upper limit of spore width and among them was the culture from Florida.

In relation to mean widths it is evident that the differences among P1, P3 and P17 may be attributed to chance. Here again as in the case of the mean lengths, the cultures from Porto Rico fall together under one class, the differences between these and P216 being significant enough. The mean of check culture P18 is also significantly different from either P216 or any of our cultures.

Considering the means of lengths and widths together, we find the dimensions as follows: P1, 46.653 × 38.754 microns; P3, 49.822 × 39.291 microns; P17, 49.197 × 39.177 microns; and P216, 42.799 × 33.834 microns. The Florida black-shank fungus is given by Tisdale and Kelley (12) as measuring 34.44 × 26.18 microns, which is a lower figure than that obtained by us. They give the average dimensions for the Java strain as 38.2 × 28.98 microns and for the Holland strain 36.67 × 25.30 microns.

These writers assumed their pathogene to be a distinct strain of *Phytophthora nicotianae* Breda de Haan. They based their assumption on "slight differences in the morphological and physiological characters".

Rosenbaum (9) in studies of the genus Phytophthora, made use of the ratio of length to width of sporangia in the separation of species. Our results on such ratios in the cultures (see table V) under study, show they are not of much value in the delineation of possible strains or lines. Thus, P216 which, according to the mean lengths and widths of sporangia, stands in a class by itself, here would seem to be equal to P3. P18, the Phytophthora from tomato, has ratios almost similar to the preceding. P1 and P17 have equal ratios.

TABLE No. V

Shows lowest, highest and modal ratios of lengths to widths of sporangia of *Phytophthora nicotianae* and an undetermined species of Phytophthora from tomato

Culture	Population	Lowest	Highest	Mode
P1	277	1:1	1.6:1	1.2:1
P3	283	1.1:1	2.5:1	1.3:1
P17	206	1:1	1.6:1	1.2:1
P216	203	1.1:1	2.5:1	1.3:1
P18	145	1:1	2.6:1	1.3:1

Summarizing the results of measurements of sporangia one is inclined to regard the Porto Rican cultures P1, P3 and P17 as probably the same strain. P216 (the Florida pathogene) differs somewhat from ours and we shall tentatively call it a different strain. Both should be placed as strains of *Phytophthora nicotianae* Breda de Haan.

Size of Chlamydospores.—Rosenbaum (9) in his investigation of members of the genus Phytophthora, among them *P. nicotianae*, says:

"As in the case of conidia, great variation in size occurs within the species," etc. However, he does not give any data on the variation in size of the chlamydospores within any particular species. The problem appears then to be, how much value may be placed on differences obtained in spore measurements in populations of any two or more pedigree lines within a definite species.

The mean diameters of chlamydospores of the same cultures (lines) employed above follow. The population in these varied from 189 to 233. P1: 35.003 ± 0.292; P3: 35.302 ± 0.387; P17: 40.087 ± 0.549; P216: 31.038 ± 0.318 and P18: 29.344 ± 0.287.

The difference in mean diameter of P1 and P3 is not significant. That between P216 and P1 is very significant, about 9 times its probable error. That between P216 and P3 is about 8.5 times its probable error; between P216 and P17 it is about 14 times its error, while that between P17 and P3 is about 7 times the error. All of the latter are significant. It is of interest to note that the difference between P216 and P18 is only about 4 times its error. If much reliability were placed on the size of chlamydospores in this genus one would at least regard P1 and P3 as the same strain but different from P17 and P216 which would again appear to be different from each other and should logically be considered distinct strains. However, that this should not be done is shown by cultural characteristics and pathogenecity studies which are given later.

Rosenbaum (9) held that "the separation and relationship of species should be made on the aggregate of characters", although he laid particular stress on morphological characters.

Leonian (6), on the other hand, regards morphological characteristics as of minor importance and has offered a key for the separation of the species of the genus Phytophthora based on physiological reactions. In his own words: "The average of all morphological, pathological and physiological features should form the specific sphere".

The delineation of strains within a species of this genus seems

to offer similar difficulties. Size of reproductive bodies alone does not seem to furnish a valuable means of separation. We have tentatively regarded P1, P3 and P17 as one strain and P216 as another strain of *P. nicotianae*. We shall now see how these behave in other reactions and how true this assumption holds.

Cultural characteristics.—The behavior of the cultures of Phytophthora, used in this work toward certain media is given below. Measurements of diameter of colonies were taken in every case nine days after planting on the media. The culture dishes employed were of a 90 × 10 mm. size. (See table VI.)

TABLE No. VI

Growth of various cultures of *Phytophthora nicotianae* and Phytophthora sp. (P 18) on different media.

Culture	Medium	Reaction
P1	Lima bean juice agar (a)	Colony covering whole surface of substratum. Aerial mycelium reaching lid of dish. Heavy white growth.
P3	Lima bean juice agar (a)	Colony growth like P1, but for aerial mycelium which is less abundant.
P4	Lima bean juice agar (a)	Essentially like that of P1.
P18	Lima bean juice agar (a)	Heavy white growth extending over entire dish.
P216	Lima bean juice agar (a)	Colony spreading over entire dish. Aerial mycelium loose, fluffy.
P1	Lima bean (juice extracted) agar (b).	Mycelium much more abundant than on lima bean juice agar. Colony covering whole bottom of dish
P3	Lima bean (juice extracted) agar (b).	Like P1.
P4	Lima bean (juice extracted) agar (b).	Like P1 and P3.
P17	Lima bean (juice extracted) agar (b).	Like P1.
P18	Lima bean (juice extracted) agar (b).	Very heavy, thick growth over entire dish.
P216	Lima bean (juice extracted) agar (b).	Slightly less growth than P1.
P1	Malt syrup agar (c)	Colony 1" in diameter, aerial mycelium scanty.
P3	Malt syrup agar (c)	Colony 1¼" in diameter Otherwise like P1.
P4	Malt syrup agar (c)	Colony 1⅛" in diameter. Other characters like P1 and P3.
P17	Malt syrup agar (c)	Colony 1 1/16" in diameter. Aerial mycelium scanty.
P18	Malt syrup agar (c)	Colony 2" in diameter. Aerial mycelium scanty.
P216	Malt syrup agar (c)	Colony 1 1/8" in diameter. Aerial mycelium present; stands out clearly; unlike P18.
P1	Malt syrup agar (d)	Colony 1⅛" in diameter. Little aerial mycelium.
P3	Malt syrup agar (d)	Colony 1 3/8" in diameter. Little aerial mycelium.
P4	Malt syrup agar (d)	Colony 1½" in diameter. Little aerial mycelium.
P17	Malt syrup agar (d)	Colony 1 1/16" in diameter. Little aerial mycelium.
P18	Malt syrup agar (d)	Colony 2 2/16" in diameter. Little aerial mycelium.
P216	Malt syrup agar (d)	Colony 1 1/16" in diameter. More aerial mycelium than P1, P3 or P4
P1	Oatmeal agar	Colony covering entire surface of substratum. Loose mycelial growth.
P3	Oatmeal agar	Like P1.
P4	Oatmeal agar	Like P1 or P3.
P17	Oatmeal agar	Like P1.
P18	Oatmeal agar	Slightly heavier growth than P1.
P216	Oatmeal agar	Like P1.

(*a*) Lima bean juice agar. Made by boiling 60 gms. of lima beans in one liter of water for 2 hrs. allowing the juice to boil down to 500 cc. after filtering. To this 10 grams of agar-agar were added and then the medium prepared in the usual manner.

(*b*) Lima beans (juice extracted) after filtering the juice for the above medium the beans were boiled a second time in 500 cc. of water adding 10 grams of agar-agar. This was not filtered.

(*c*) Malt syrup agar. Prepared like Leonian's (6) medium No. I except that instead of the dry malt extract we used malt syrup.

(*d*) Prepared like *c* but with 10 grams of malt syrup instead of 5 grams.

These pathogenes did not fructify well in any of the media. Non-sporulating 10-day-old cultures on oatmeal agar were set aside. The fruit fly (*Drosophila amphelophila*) laid eggs in them and larvae were found in the substratum a few days later. An examination of the cultures at this time (cultures 18 days old), revealed the presence of numerous sporangia and chamydospores. A few oospores were also encountered. Measurements of the three types of spores were made for taxonomic purposes.

Summarizing the results which appear in table VI, it may be concluded that the differences between the different cultures on the various media are not enough to warrant a safe separation of the same into different strains or species. It appears, however, that P18 differs enough from all the others to warrant its designation as a distinct form. This will be confirmed with additional data on other reactions. All the other cultures are much the same in their reactions on the above media.

Pathogenicity. The pathogenicity of cultures was tested in various ways. In every case *Pythium* cultures which cause damping-off in the seedbeds were employed as checks. In the following paragraphs are presented the details of the pathogenicity tests.

Method for inoculation of plants in the field and those growing in pots of sterilized soil.—Six plants were inoculated with each one of the cultures. The basal part of each plant was sterilized with mercuric chloride solution (1–1000) and then washed with boiled distilled water. One of the "sand" leaves from each of three plants of each set was cut at the axil and a wound made at this place into the tissues of the stem. Here the inoculum (a small bit of mycelium from 7-day-old oatmeal agar cultures) was placed. The wound was then covered with a little absorbent cotton, and the whole wrapped with a piece of heavy paper. The cotton was moistened with boiled water soon afterwards and on the following day. The remaining three plants in each set were similarly wounded and inoculated but this time away from the axil of the leaf.

Test No. 1—February, 1927. "Borinquen" (Connecticut Round Tip) plants growing in pots were used. They were healthy and vigorous and the flower buds were opening at the time of inoculation. They were inoculated with the cultures P1, P2, P3, P4, P10 and P14. The P2 used here is a culture of *Pythium de Baryanum,* isolated from tobacco seedlings.

On March 4, 1927, all plants inoculated with P1, P3, P4, P10 and P14 had wilted. The wilting of the leaves was more complete and pronounced on the side of the plant in which the inoculation was

performed. Browning and blackening had advanced up and down the stem considerably, about 6″ above the wound and about 2″ below it. The symptoms were like those occurring in the field under natural conditions. The plants inoculated with P2 showed no evidence of infection; only a slight browning or discoloration of the bark was apparent; but below it the wound healed and the plants continued their normal growth like in the check plants where no inoculum was placed in the wounds.

Test No. 2—Test No. 1 was duplicated on March 9 on plants of the "País" type of filler tobacco with similar results.

These tests proved the ability of P1, P3, P4, P10 and P14, to produce black-shank and the non pathogenicity of P2 in relation to this disease. It was also demonstrated how varieties which are resistant under natural field conditions succumb to the disease when artificially inoculated.

Test No. 3, December 23, 1927. Healthy plants of the Porto Rico filler ("País") and "Borinquen" varieties from a field known to be free from the pathogene (in the Experiment Station grounds) were inoculated when about 18″ high. The cultures used were P1, P2, P3, P4, P10, P14, P16, P17, P18 and P20. The notes gathered on December 25 showed infection had been produced by any of the cultures except P2, P16, P18 and P20 as indicated by a pronounced brownish discoloration around the wounds in the former and only a slight discoloration in the latter. The results of subsequent observations are tabulated below.

TABLE No. VII

Results of inoculations of "País" and "Borinquen" plants with various Phythophthora cultures.

Culture	December 27 Leaves wilting		December 31 Plants wilting		January 4
	"Borinquen"	"País"	"Borinquen"	"País"	
P1	5 plants	4 plants	6	5	All dead
P2	No plants	No plants	None.....	None.....	All healthy
P3	3 plants	3 plants	6	6	All dead
P4	4 plants	4 plants	6	6	All dead
P10	6 plants	5 plants	6	5	All dead
P14	4 plants	4 plants	6	6	All dead
P16	No plants	No plants	None.....	None.....	All healthy
P17	6 plants	6 plants	6	6	All dead
P18	No plants	No plants	None.....	None.....	All healthy
P20	No plants	No plants	None.....	None.....	All healthy

The only difference between the reactions of the two varieties to the different cultures was a more rapid wilting of the "Borinquen" plants.

The wounds of plants inoculated with the remaining cultures were

examined by removing the paper and cotton. All wounds had healed in those plants inoculated with P2, P16 and P20, while those inoculated with P18 showed a slight browning which progressed for about ¼″ beyond the cut cortex but there it stopped and the wound healed.

Test No. 4; December 23, 1927: Healthy plants of the "País" and "Borinquen" varieties grown in pots in sterilized soil were inoculated with P216 and P217 cultures at the time of the opening of the first blossoms. The plants were kept in the greenhouse. Six plants received the P216 inoculum, six the P217 and six remained as checks in each case. The results at the end of nine days (Dec. 31) showed P216 infected all the six plants of each variety, while those inoculated with P217 were unaffected as were also the check plants. The Florida organism (P216) is also able to produce black-shank in Porto Rico.

It should be noted that one of the varieties used in the test, namely, the "País" was previously given as second in degree of resistance in Porto Rico.

That the black-shank pathogene is the cause of a damping-off of tobacco seedlings was proved by the following test and by others which will be given later in another publication on damping-off diseases.

Test No. 5. Small tobacco seedlings and *Ricinus communis* plants about 6″ high, growing on sterilized soil in the same pots were inoculated with each of the organisms on April 25, 1928, by transferring small bits of mycelium from 8-day-old oatmeal agar cultures to the growing tip and one leaf of each of two tobacco plants in each pot. A needle prick was made on the leaf blade, leaf midrib and bud for this purpose, and after the inoculum was placed in these wounds, the part was covered with cotton and moistened. All the pots were covered with bell jars which had been previously sterilized by washing with a concentrated solution of mercuric chloride. The cultures employed were P1, P2, P3, P4, P16, P17, 18P, P20, P16 and the culture from Sumatra obtained through Mr. C. M. Tucker. Daily observations were made until May 5th when the experiment was completed.

P1, P3 and P4 produced an early severe infection on the leaf, stem and bud. The discoloration was slightly paler than that produced by P17. Infection proceeded from the inoculated buds down to the base of the seedlings. Leaf infections occurred. The mycelium appeared to extend from the infected parts through the soil into neighboring tobacco and *Ricinus communis* seedlings which it killed.

Portions of diseased parts were examined daily for sporangia. It was found that under the conditions of the experiment few sporangia were formed, but the mycelium was abundant. Finally all the tobacco and *Ricinus* plants were killed.

The results with P17, P216 and the Sumatra culture were similar to the preceding except for the discoloration of the tissues which seemed darker with P17. Infection was slower in the case of the Sumatra culture.

With P2, P16 and P20 none of the large tobacco seedlings showed infection, while all the small ones readily succumbed to the disease. *Ricinus* seedlings reacted similarly to these cultures.

In the case of the seedlings inoculated with culture P18 there were a few small seedlings killed and only a small spot produced on an inoculated leaf on a larger seedling. This leaf spot finally ceased to increase in size. It did not seem of much significance.

This experiment proves that cultures P1, P3, P4, P17, P216 and the Sumatra culture (all *Phytophthora nicotianae*) produce both damping-off of tobacco and *Ricinus* seedlings and the black-shank disease of tobacco. The fact that the Sumatra culture did not produce as rapid infection as the others may be due to the age of the culture. It further shows that small seedlings of tobacco and *Ricinus communis* are killed when inoculated with P2, P16 and P20 (*Pythium de Baryanum*) and that large plants remain uninfected. Culture P18 (an undetermined species of *Phytophthora* from tomato) may cause a mild case of damping-off under certain conditions but is usually non-pathogenic to tobacco.

Test No. 6—Potted plants of two Porto Rican varieties of eggplant were inoculated with *Phytophthora nicotianae* cultures (P1, P3, P17, P216 and Sumatra), *Pythium de Baryanum* cultures (P16 and P20) and the undetermined Phytophthora sp. P18, on April 25, 1928. At the time of the inoculation these plants had reached the stage at which they start to send out the first flower bud.

Inoculations were made in each plant at the terminal bud and at the axils of the leaves. The terminal bud was injured about ¼" below the unopened leaves and here the inoculum was placed. The wound was covered with absorbent cotton moistened with sterile water and covered with a glassine bag which was held in place with a gem paper clip. The cotton was kept moist for the first three days. At the axil of one leaf in each plant a needle prick inoculation was performed. A piece of absorbent cotton was wrapped around the stem and petiole and over the axil. This was also kept moist for three days. The two varieties of eggplant ("Fajardo") and ("Ca-

muy'') here employed are non-commercial, but show a remarkable resistance toward the bacterial wilt. Two plants of each variety were inoculated with each of the following: P1, P3, P16, P17, P18, P20, P216, and the Sumatra culture and two plants kept as checks.

Observations were made every day. The experiment was completed on May 3rd. The results were as follows:

P1 produced infection in the bud of the ''Fajardo'' plants while in the ''Camuy'' plants infection occurred in both buds and leaves. The fungus made a more rapid advance into the tissues on the ''Camuy'' plants than in those of the ''Fajardo'' variety. A similar effect was produced by P3, P17 and P216. The Sumatra culture also produced infection but slowly.

P16 and P20 produced a slight infection which soon stopped. P18 caused the death of the buds in both varieties and wilting of the leaves in the ''Camuy'' variety alone.

The results prove that the two varieties of eggplant, ''Camuy'' and ''Fajardo'' are susceptible to infection by *Phytophthora nicotianae* (P1, P3, P17, P216 and Sumatra culture) and slightly susceptible to the *Phytophthora* sp., P18, when these plants are wounded and the inoculum is transferred to the wounds. Under the same conditions *Pythium de Baryanum* (P16 and P20) did not seem to be able to produce vigorous infection. The ''Camuy'' variety appeared to be slightly more susceptible to *Phytophthora nicotianae* than the ''Fajardo'' variety.

From the studies on pathogenicity of P1, P3, P4, P10, P14, P17, (Porto Rican strains), P216 (Florida strain) and the Sumatra culture all are pathogenic toward tobacco. There are no differences that would indicate them to be different strains, unless the slightly slower development of the Sumatra organism be so considered. It has already been pointed out that this may be accounted by the age of the culture.

Size of sporangia alone, then, is left as the only means in these studies to separate strains, if these exist. If Tisdale and Kelley's (12) assumption that their pathogene is a strain of *P. nicotianae* is valid we propose that the black-shank pathogene from Porto Rico be regarded as a distinct strain of the same species.

LIFE HISTORY

The life history of *P. nicotianae* involves a sexual stage and two spore forms of an asexual stage.

The primary cycles appear either as damping-off and lesions on seedlings in the beds or as lesions on roots and stems in large plants

in the field. They may also take the form of a swelling up of the transplants before any roots are developed.

PATHOGENESIS

Inoculation.—The main sources of inoculum are infested debris from plants left over from the preceding crop and infested manure. In all probability, the mycelium vegetates in those decomposing plant parts and crops of sporangia and conidia are produced from it at intervals. Again the primary cycles may be induced by the mycelium without the production of conidia or sporangia. It is also possible that germinating chlamydospores which are found in the soil in abundance, may produce an invading mycelium. Oospores also present in the soil and debris probably constitute an appreciable part of the inoculum.

Incubation.—Oospores have not been seen germinating but they probably give rise to a primary mycelium which in turn gives rise to invasion hyphae and sporangia or conidia. Chlamydospores have been seen to germinate by sending a number of germ tubes from their surface. Rosenbaum (9) failed to obtain germination of chlamydospores of *P. nicotianae.*

Germination tests of sporangia or conidia in Van Tieghen cells show that when mature these germinate readily. Sporangia from three-week-old oatmeal cultures were employed. In the early stages of germination the contents of the sporangia are quite dark and in a short while they seem to break up into spherical masses. (Fig. 1). Shortly afterwards movement is observed inside. The fine membranes at the papillate ends break and the zoosporic masses begin to ooze out through the opening. The zoospores remaining inside the sporangium begin to move about violently until all have succeeded in reaching the papillate end and then make their way to the outside, leaving empty sporangial shells. The writer has not observed zoospores germinating *in situ* as seen by Tisdale and Kelley (12) for this species and by other writers for other species. These zoospores play the most important rôle in the production of secondary cycles. They germinate by means of germ tubes. Conidia germinate also by sending out one or several tubes. The contents of the conidia pass into the primary mycelium and finally the shell breaks up and disappears.

The infecting hyphae from the mycelium in the soil or that produced by chlamydospores and oospores and the germ tubes from conidia or zoospores, enter through the stomata, or penetrate through wounds made on the stems and roots.

Infection.—The invading hyphae o tubes penetrate the epidermal cells and from here the branching hyphae extend into the underlying cell layers. The cortex of stems and roots is soon destroyed. Infection is very rapid under extremely moist conditions combined with favorable temperature. The hyphae cause the destruction of the tissues in a short time. On the diseased parts, mainly spots on leaves and stem lesions at the surface of the soil, sporangia are abundantly produced. During cloudy weather and specially in low places they will be found in large numbers in the water which collects in small depressions in the ditches, such as are made by the heels of shoes. They are transferred to suscept parts and start the secondary cycles.

SAPROGENESIS

Phytophthora nicotianae lives in the soil as a saprophyte on plant debris, old tobacco stems or manure. It develops actively in these media and readily spreads through the soil. During its saprophytic existence it forms chlamydospores and oospores. In fact, it is not difficult to find oospores in the soil where the fungus is present.

Secondary cycles are initiated by conidia produced in the primary lesions.

PATHOGENESIS

Inoculation.—Sporangia, conidia and zoospores are carried by irrigation water or by water running down the ditches during rains to the suscept parts, lower leaves, stems and roots. They may be transported there by other agents, like tools, workmen, animals, etc. A common means of transportation of the inoculum is by laborers who perform the weeding of the seedbeds. Spattering rain usually carries the inoculum over to the surface of the leaves. After the inoculum reaches the suscept parts it produces infection in the manner described for the primary cycles.

EPIPHYTOLOGY

According to Tisdale and Kelley (12) the minimum soil temperature for the black-shank fungus is probably below 20°C. Unfortunately, we are not equipped to make soil temperature experiments. Consequently, the determination of the range of temperature for this pathogene could not be made. All that we can give at this time is merely based on observations made during the last two years. As has previously been pointed out, an outbreak of black-shank occurred in two fields of tobacco in the summer of 1927. This is the period of highest temperatures. On the other hand, the disease is

prevalent throughout the tobacco growing season, October to March. Plants inoculated at all times of the year have contracted the disease if moisture was adequate. The fungus will cause damping-off of seedlings in any month of the year, provided the soil is kept moist. Moisture, then, is the most important and dominating environmental factor in Porto Rico. In years where there are frequent but not heavy rains there are fewer cases of the disease. Moisture seems essential for the production of sporangia, for their germination and for dissemination of the inoculum in general. Standing water is especially favorable for spores to germinate.

CONTROL

According to Tisdale (10), "the black-shank organism persists in the soil at least five or six years after tobacco culture has been discontinued on infested land". This makes the possibility of control through rotation of crops rather unfeasible. In Porto Rico the best tobacco lands are kept under tobacco culture year after year and it seems unlikely that this practice will be abandoned in the future. We must seek then, some other means of control, either preventive or eradicatory. Eradication through removal of diseased plants from fields as soon as they are detected is always a good practice to follow. Once plants become infected it is the only safe thing to do. Seedlings which show lesions as described in previous paragraphs should be destroyed. Such plants when transplanted will sooner or later succumb to the disease. Transplants should be scrupulously selected. The eradicatory measures for the damping-off produced by *P. nicotianae* will be discussed in another publication.

Tisdale and Kelley (12) consider the steaming of soils as impractical because of the high cost. They report a case in which the treatment was only effective for one season. In Porto Rico absolute sterilization to last for more than one season would be improbable. Drainage and irrigation water will carry the inoculum from one field to the other.

Preventive measures may be of some value. The treatment of plants with chemicals to protect them from black-shank may not be altogether practical. Tisdale and Kelley (12), using Semesan at the rate of 80 lbs. to the acre and inoculated sulphur at the rate of 360 lbs. to the acre were able to keep the disease in check on transplants up to about 30 days; but at the end of 90 days all the plants were diseased in the latter and about 99 per cent in the former case. Tisdale had already reported in 1923 (10) negative results in treatments of plants in rows with 2–2–50 and 4–4–50 Bordeaux mixture.

We have made tests with 5–5–50 Bordeaux mixture on "Ceniza" plants in pots. The soil for these was prepared with about 50 per cent manure and 50 per cent loam. All pots were first infested with actively-growing cultures of *P. nicotianae* (Porto Rican isolations). In one set of 25 pots the treatment was made a week before the seedlings were planted and a second set of 25 plants, at the time of planting. Notes were taken at short intervals. On November 24, 1927, treatment of the first set of plants was made by soaking the soil well with the mixture. The soil was again soaked fifteen minutes later. A week later, healthy seedlings were planted in the pots of both sets and the first treatment of the second set made. At the end of the second week a second application of the mixture, equivalent to about 1 gallon per square foot of soil surface, was applied in the pots of the plants which were still healthy. By January 4, 1928, forty-eight plants in both sets had died and the two remaining ones showed typical black-shank symptoms.

From these results it appears that two treatments of Bordeaux mixture of as high a formula as 5–5–50 does not prevent black-shank in infested soils. No injury was produced on the stem or leaves by the Bordeaux mixture. Tisdale (10) reports injury to the lower leaves by a 4–4–50 Bordeaux mixture.

There is another way of preventing the disease. When healthy seedlings are pulled from fields where there is an infection of damping-off the seedlings should be dipped in Bordeaux mixture (4–4–50 or 5–5–50). That this treatment is effective has been proved in the following experiment. Three hundred apparently healthy plants from a seedbed with infection of seedling rot or damping-off were dipped for one minute in a 4–4–50 Bordeaux. They were planted in a garden (supposed to be free from the pathogene). Three months later there was not a single plant with any symptoms of the disease. The bed from which the seedlings were taken had been previously infested with the black-shank pathogene and slightly protected by an unsuccessful copper treatment.

Control by breeding and selection of resistant strains of tobacco.— In Florida, Tisdale and Kelley (12) report progress made in the development of resistant strains of Big Cuba Tobacco. Selection and crossing experiments have been made in Java with a similar aim, D' Angremond (4).

We have looked toward the breeding of tobacco varieties in Porto Rico as the most effective means of attacking the disease problems. However, work in tobacco has certain aspects of its own. A plant

whose commercial value is dependent entirely on the quality of its leaf is certainly difficult to handle. The breeder naturally faces great difficulties. To think that the quality of a plant part so variable as the leaf is, must possess a certain degree of uniformity, and that this "quality" consists of desirable color, texture, taste, burn and aroma. Again the "tastes" for a certain color are also variable. The demand for different shades of color in the wrapper may be different in different years. Then to go back to the environment and realize how susceptible is the tobacco plant to it, that "quality" must vary with the type of soil, fertilization, intensity of sunlight, altitude and the region. Other factors, are not considered here which would apply to all types of tobacco.

The problem then in Porto Rico would be which variety to grow for a certain soil and region and with the desirable resistance to *P. nicotianae* together with the essential qualities enumerated above.

Among the Porto Rican types of tobacco there is one which shows a high resistance to black-shank as already stated. Fortunately, this type (the "Puerto Rico" wrapper) has been properly selected by the Porto Rican Leaf Tobacco Co. This type has given excellent results in the 1927–28 crop. Promising results have been shown by other resistant strains of tobacco, originated from crosses made by that Company in recent years. There is every indication that these selected strains will go a long way toward solving the disease problem for the moment. Work must be continued in the selection of those types.

When our preliminary observations early in the season in 1926 showed us that there were certain varieties of tobacco which normally would not show much black-shank, it was arranged to start some crosses between those and other more desirable, but susceptible varieties or types.

Crosses were begun that year and the first generation was grown in the summer of 1927, followed by the second generation in the fall of the same year. Crosses were as follows:

"Vuelta Abajo" × "Borinquen"	(direct and reciprocal)
"Vuelta Abajo" × "Ceniza"	(direct and reciprocal)
"Vuelta Abajo" × *Consolation*	(direct and reciprocal)
Expt. Station × "Borinquen"	(direct and reciprocal)
Expt. Station × "Ceniza"	(direct and reciprocal)
Expt. Station × *Consolation*	(direct and reciprocal)
Expt. Station × "Vuelta Abajo"	(direct and reciprocal)

The *Expt. Station* × "Borinquen" crosses were discarded in the first generation because of high susceptibility to black-shank.

Some apparently good individuals were selected in the second generation of the remaining crosses. They were selfed and the seed has been kept for the third generation to be grown in the fall of 1928.

SUMMARY

1. A disease known in Porto Rico as "pata-prieta" and probably the same as the black-shank from the Southern United States and the "Lanas" or "bibit" of the Dutch East Indies has been destructive in Porto Rico during the last few years on tobacco.

2. The pathogene which is responsible for this disease may also attack *Ricinus communis*, potato plants, pepper, tomato and eggplant seedlings under certain conditions in Porto Rico.

3. A non-commercial variety of tobacco (mammoth-type) proved to be the most susceptible of all varieties to the malady. Of the two important commercial cigar-wrapper varieties in Porto Rico, one was found to be very susceptible and the other very resistant; while all the cigar-filler varieties were slightly susceptible. New strains of a Porto Rican variety of cigar-wrapper tobacco have exhibited a high resistance.

4. First generation populations of crosses between a very susceptible variety and other susceptible or slightly resistant types were very susceptible to the attacks of the pathogene. On the other hand, those crosses of resistant varieties with susceptible ones were more resistant than the susceptible parent.

5. The symptoms of the disease are produced on plants of all ages. A bed rot and seedling blight, a rotting of transplants, a blackening of the basal parts of the stems of plants of all ages and a leaf spot on big plants, are the most important morphological symptoms.

6. The causal organism also produces a severe rotting of seedlings in the seedbeds.

7. The black-shank pathogene was the cause of a disease of transplants called "hinchado" (water-soaked or swollen).

8. The causal pathogene is here given as *Phytophthora nicotianae* Breda de Haan. The organism from Porto Rico appears to be morphologically different from that of Florida. The Porto Rican fungus is, therefore, considered a different strain. The Porto Rico and the Florida strains were not compared culturally or morphologically with the strain from Sumatra.

9. The Forida strain does not differ from the Porto Rican strain essentially in cultural characteristics or in pathogenicity.

10. The size of chlamydospores is of no value in the separation of the strains. Use was made only of the size of sporangia.

11. Moisture is a dominating factor in outbreaks of damping-off caused by the black-shank pathogene.

12. Irrigation water is an important agent in the transportation of the pathogene from one field to the other.

13. *P. nicotianae* lives in the soil as a saprophyte on plant debris, old tobacco stems or manure.

14. As control measures are recommended: (*a*) the removal of diseased individuals, (*b*) the rigid selection of seedlings before setting out in the field, (*c*) the dipping of healthy seedlings obtained from a diseased bed in a 4–4–50 or a 5–5–50 Bordeaux mixture before transplanting, (*d*) the selection and breeding of resistant varieties.

15. The treatment of the soil with Bordeaux mixture was not effective in preventing the disease.

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EXPLANATION OF PLATES

PLATE VI

Fig. 1. Sporangia of P3.
Fig. 1–A. Sporangia of P3 germinating.
Contents breaking up into spherical zoosporic masses.
Fig. 1–B. Empty sporangia shells.
Fig. 2. Sporangia of P 17.
Fig. 3. Sporangia of P 1.
Fig. 4. Sporangia of P 216.
(Sporangia essenially alike.)

PLATE VII

Fig. 5. Chlamydospores of P3.
Fig. 6. Chlamydospores of P 1.
Fig. 7. Chlamydospores of P 17.
Fig. 8. Chlamydospores of P 216.
Fig. 9. Oogonia and Antheridia of P17.
Fig. 10. Oospore of P17.
Fig. 11. Oospores of P3.
Fig. 12. Oospore of P 216.

PLATE VIII

Fig. 13. Mycelium of P3.
Fig. 14. Mycelium of P216.
Fig. 15. Mycelium of P1.
Fig. 16. Mycelium of P17 (Note some fertile branches).
Fig. 17. Mycelial threads in xylem cells.
Fig. 18. Epidermal cells of *Nicotiana tabacum* in tangential section showing the invading hyphae.

PLATE IX

Figs. 19, 20, 21 and 22. Lesions in seedlings. Note that infection has occurred just above the surface of the soil in figs. 21 and 22. In figures 12 and 20 infection occurred at the petioles of the leaves.
Fig. 23. A full grown plant in the field severely affected with the disease. All the leaves are yellow and shriveled.
Fig. 24. Three medium-sized plants in the field. The one on the left is completely wilted; that on the right has recently contracted the disease (note the wilted lower leaves).

PLATE X

Figs. 25, 26, 27 and 28. One-half natural size. These show the characteristic black-shank lesions at the base of the plant. Note the poor root system. The arrows point at the uppermost limit of the lesions. In figure 26, infection occurred at the leaf blade and followed down the petiole and stem.
Fig. 29. The same plant as in figure 28, much reduced. (Note shriveled condition of foliage).
Fig. 30. Same plant as in figure 26 (much reduced). Shows both the lesions at the base of the plant and the wilted leaves.

PLATE XI

Fig. 31. A field of tobacco under shade. The variety here is the non-commercial mammoth type, a very susceptible variety. The majority of the plants have contracted the disease at this stage.

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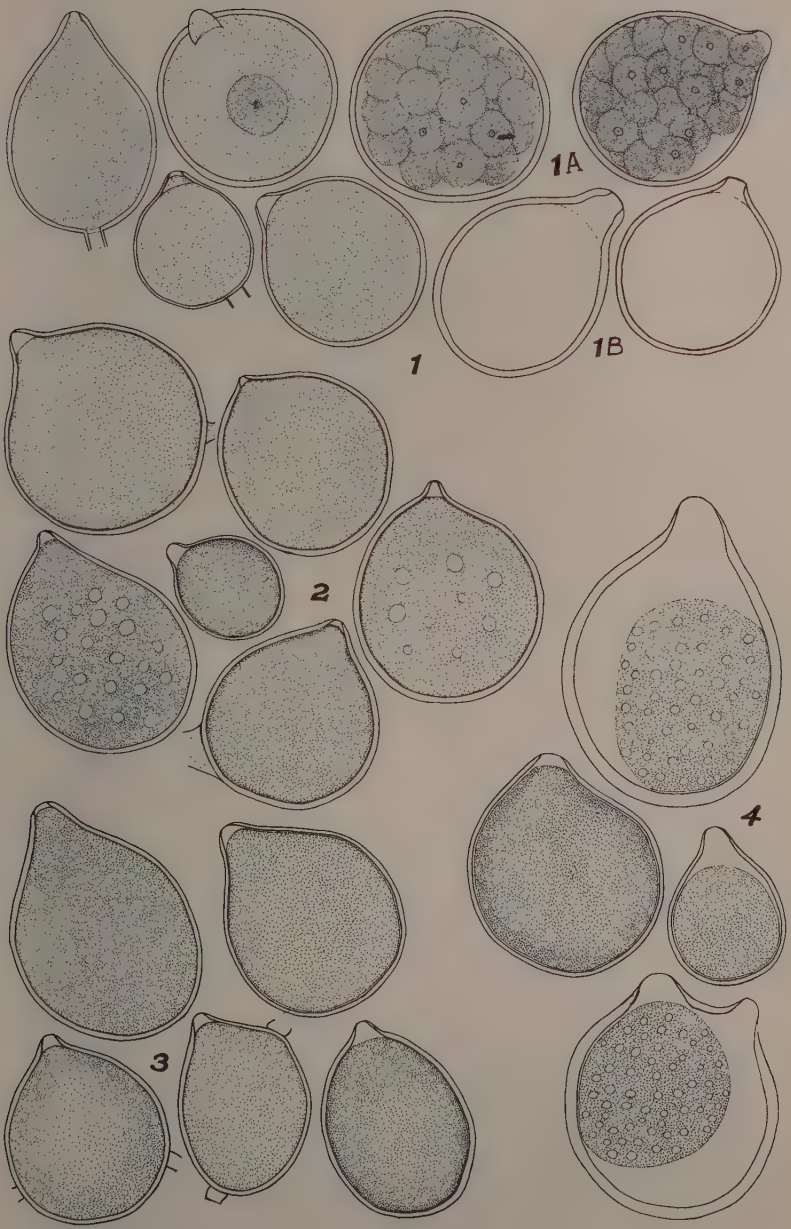
PLATE VI



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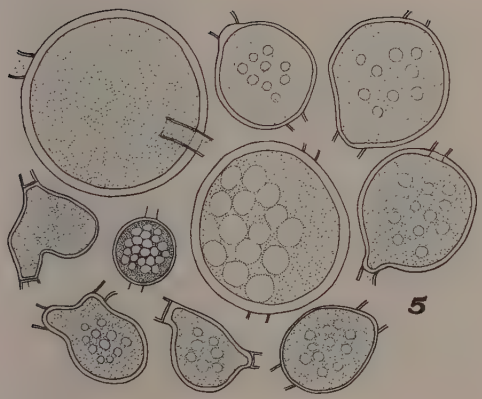

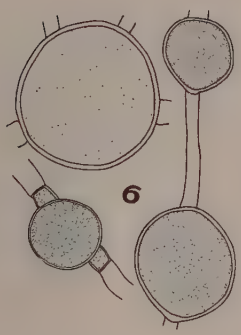

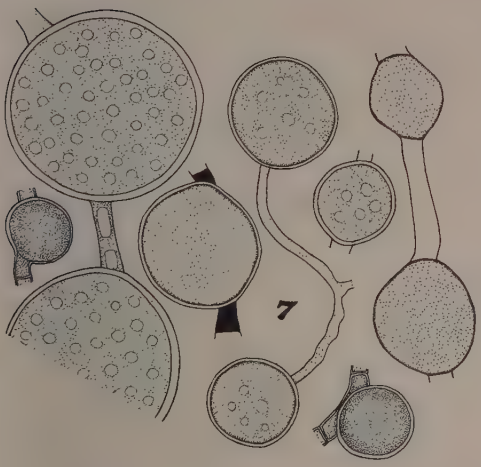

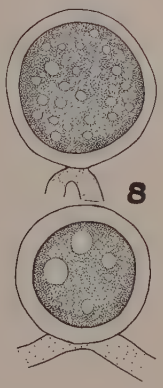

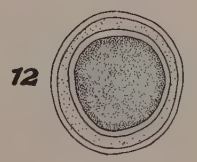

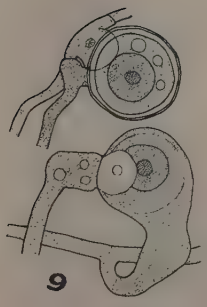

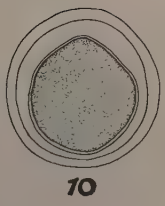

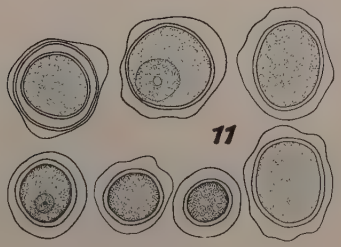

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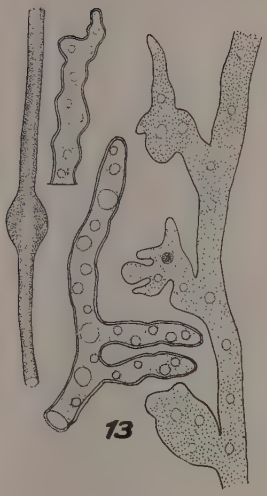
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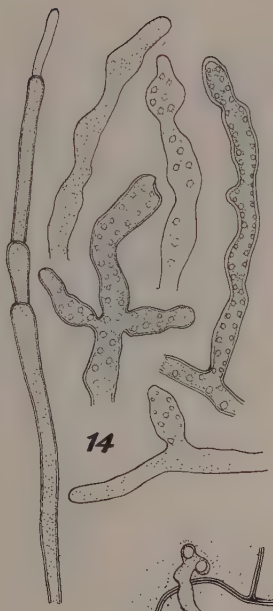
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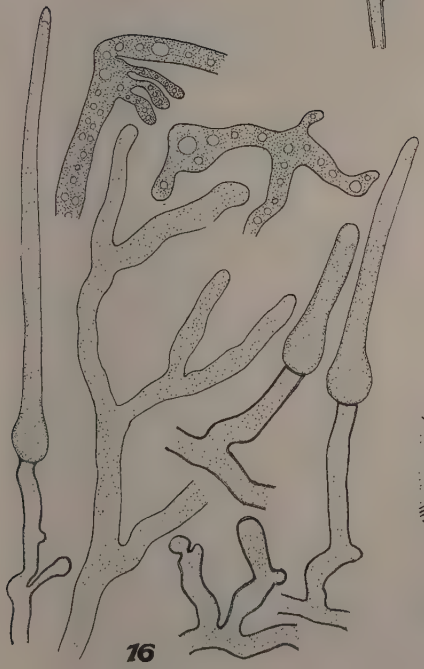
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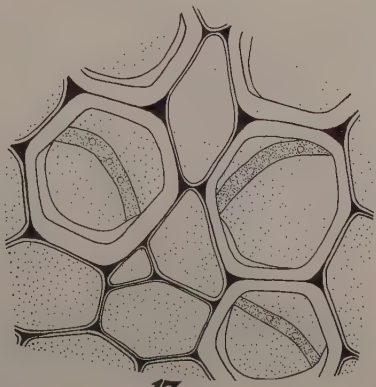
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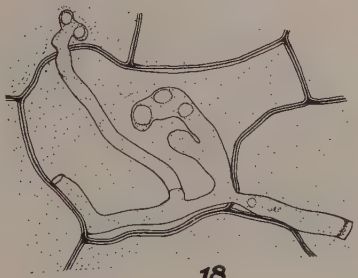
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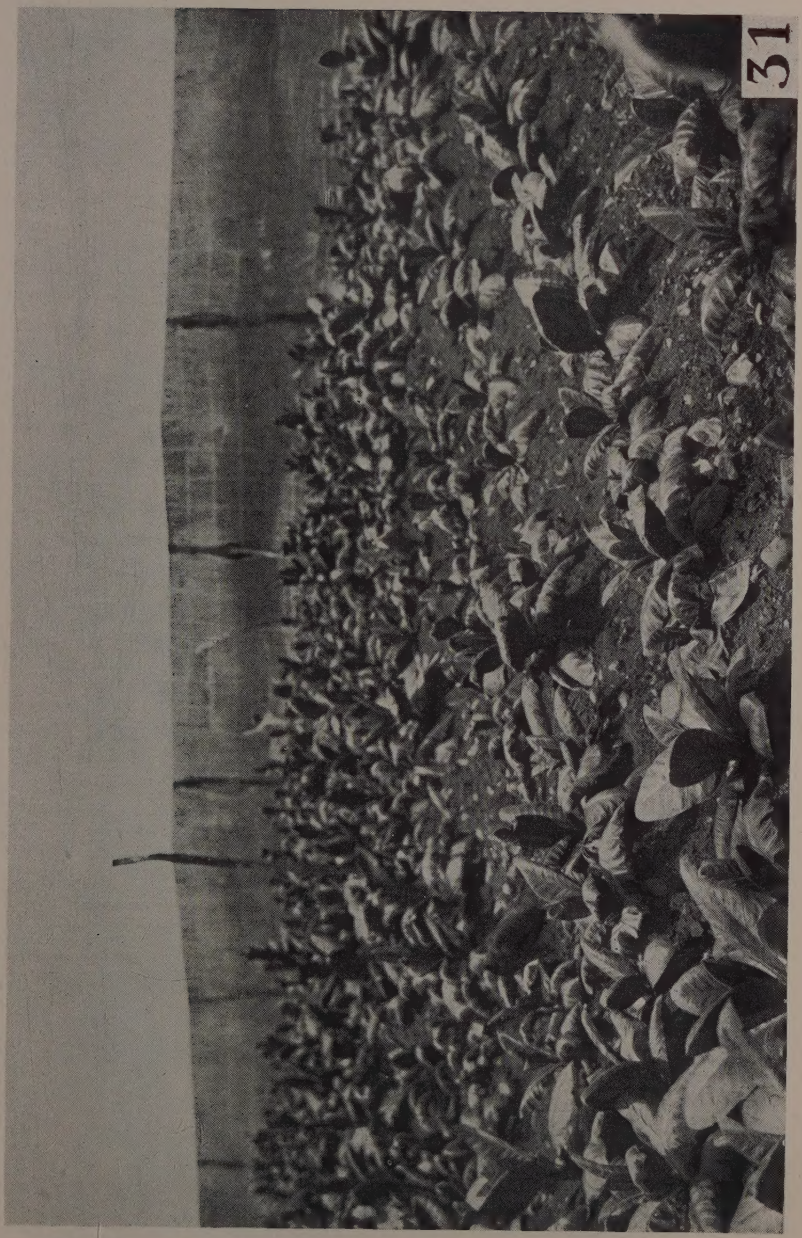
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